**Supplementary Information for:**

# Elevated monocyte phosphorylated p38 in nearby employees after a chemical explosion

**Authors:**

André Sulen, Stein H. L. Lygre, Sigrun M. Hjelle, Bjørg E. Hollund and Bjørn T. Gjertsen

**Supplementary materials and methods**

*Immunophenotyping of monocyte subsets (Fig. S1)*

PBMCs from blood collected in EDTA tubes from blood donors were isolated by density gradient separation (Lymphoprep™, Stemcell Technologies). Live cells were stained with Anti-CD45 FITC (clone MEM-28, EXBIO), Anti-HLA-DR Pacific Blue (clone MEM-12, EXBIO), Anti-CD16 BV605™ (clone 3G8, BioLegend) and Anti-CD14 biotin (clone M5E2, Biolegend) in combination with Strepdavidin Alexa Fluor® 647 (Molecular Probes). Acquisition and analysis was carried out as described in materials and methods in the publication.

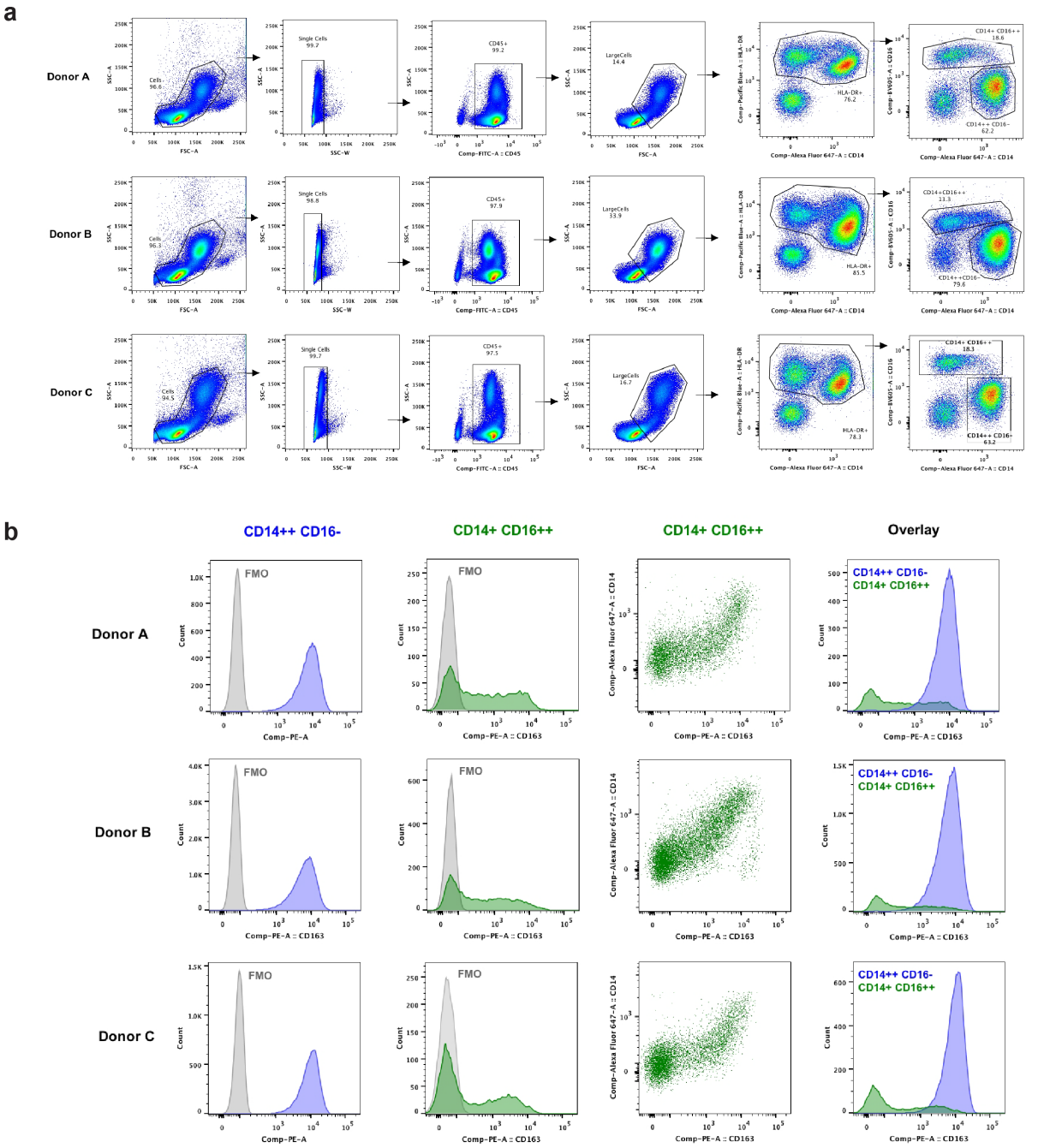


**Fig. S1.** CD163 is expressed predominantly in classical monocytes compared to non-classical monocytes. Live PBMCs from three healthy donors were stained with antibodies to evaluate CD163 expression in monocyte subsets. (**a**) Far right scatterplots display gating strategy of CD45+ HLA-DR+ cells into classical (CD14++ CD16-) and non-classical monocytes (CD14+ CD16++) based on CD14 and CD16 expression. (**b**) Histogram overlays and dot plots display CD163 expression in the monocyte subsets for each donor.

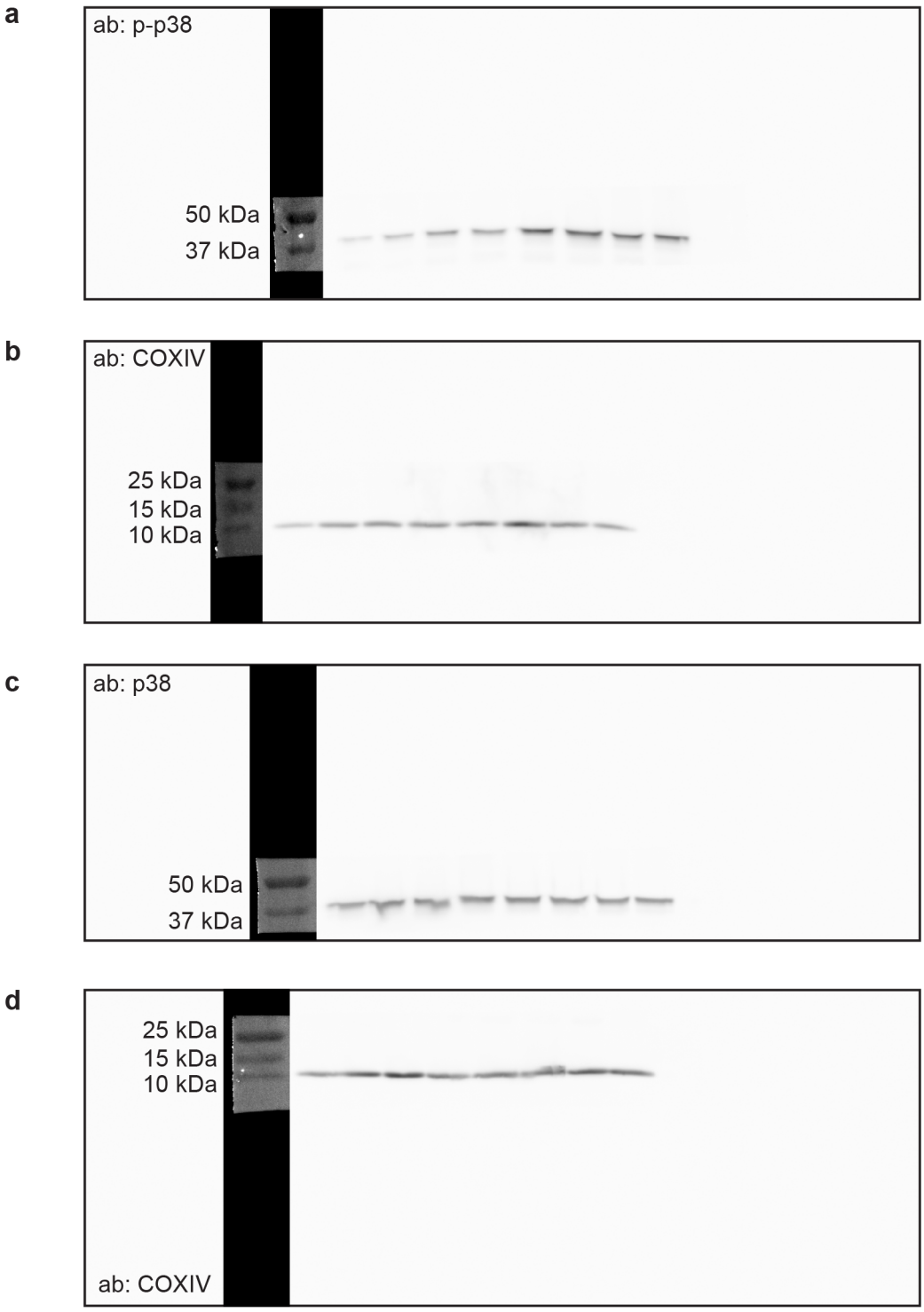


**Fig. S2.** Uncropped Western blots of PBMC lysates from eight individuals with high or low monocyte p-p38, probed with antibodies detecting p-p38 (**a**), total p38 (**c**) and COX IV (**b**,**d**). Reference ladder is overlaid at its correct position with band sizes indicated.


**Fig. S3.** Distribution of p-p38 MFIs in all predictor subgroups in regression analyses (Tables 1 & 2). Boxplots displaying p-p38 MFI values for monocytes **(a)** and T-cells **(b).** Boxes extend from 25th to 75th percentiles, whiskers display minimum to maximum values and lines in boxes represent median values.

**Table S1.** Demographics of the study group included in the regression analyses (Tables 1 & 2).

| | All n = 301 | >20 km n = 104 | <6 km n = 105 | Employees n = 92 |
|---|---|---|---|---|
| Gender | | | | |
| Female | 128 | 52 | 60 | 16 |
| Male | 173 | 52 | 45 | 76 |
| Age (years) | | | | |
| [17, 39] | 112 | 32 | 45 | 35 |
| [40, 49] | 79 | 29 | 22 | 28 |
| [50, 67] | 110 | 43 | 38 | 29 |
| Smoking habits | | | | |
| Non-smokers | 208 | 76 | 74 | 58 |
| Smokers | 93 | 28 | 31 | 34 |
| Geographic[1] | | | | |
| Inhabitant >20km | 104 | 104 | 0 | 0 |
| Inhabitant <6km | 105 | 0 | 105 | 0 |
| Employees | 92 | 0 | 0 | 92 |
| BMI | | | | |
| Low [<-, 24.9] | 106 | 31 | 49 | 26 |
| Medium [25, 29.9] | 133 | 48 | 37 | 48 |
| High [30, ->] | 62 | 25 | 19 | 18 |

1) *cf.* Figure 1a

**Table S2**. Effects on mean monocyte p-p38 from gender, age, smoking status, geographic group, body mass index (BMI) and hs-CRP (n=300). Crude mean level of monocyte p-p38 and their 95% confidence intervals (95%CI) are listed in the left part of the table, while adjusted coefficients (coef.), p-values (p) and 95% confidence intervals (95%CI) are listed in the right part of the table.

| | n (%) | Crude | | Adjusted | | |
|---|---|---|---|---|---|---|
| | | Mean | 95% CI | coef. | p | 95% CI |
| Gender | | | | | | |
| Female | 127 (42) | 15,206 | (14,007, 16,407) | ref | | |
| Male | 173 (58) | 15,207 | (14,241, 16,175) | -607 | 0.46 | (-2,224, 1,010) |
| Age (years) | | | | | | |
| [18, 39] | 112 (37) | 16,213 | (14,973, 17,453) | ref | | |
| [40, 49] | 79 (26) | 14,668 | (13,182, 16,156) | -1,415 | 0.15 | (-3,328, 499) |
| [50, 67] | 109 (37) | 14,565 | (13,333, 15,797) | -1,410 | 0.12 | (-3,172, 352) |
| Smoking status | | | | | | |
| Non-smokers | 207 (69) | 14,963 | (14,036, 15,889) | ref | | |
| Smokers | 93 (31) | 15,752 | (14,468, 17,036) | 661 | 0.43 | (-985, 2,307) |
| Geographic[A] | | | | | | |
| Inhabitant >20km | 104 (35) | 13,878 | (12,563, 15,192) | ref | | |
| Inhabitant <6km | 104 (35) | 15,397 | (14,137, 16,657) | 1,665 | 0.08 | (-169, 3,500) |
| Employees | 92 (30) | 16,497 | (15,183, 17,810) | **2,721** | **0.006** | **(796, 4,647)** |
| BMI | | | | | | |
| Low [<-, 25) | 106 (35) | 14,659 | (13,372, 15,945) | ref | | |
| Medium [25, 30) | 133 (45) | 15,261 | (14,194, 16,327) | 821 | 0.35 | (-910, 2,554) |
| High [30, ->] | 61 (20) | 16,045 | (14,184, 17,905) | 1,248 | 0.27 | (-974, 3,470) |
| hsCRP[B] | | | | | | |
| Low [0, 2] | 215 (72) | 14,904 | (14,074, 15,733) | ref | | |
| Medium [3, 5] | 52 (17) | 15,522 | (13,388, 17,657) | 422 | 0.69 | (-1,644, 2,488) |
| High [6-21] | 33 (11) | 16,690 | (14,041, 19,338) | 1,763 | 0.17 | (-782, 4,309) |

A) Described in Fig. 1a
B) 1 individual excluded due to missing CRP data.

**Table S3**. Effects on mean T-cell p-p38 from gender, age, smoking status, geographic group, body mass index (BMI) and hs-CRP (n=300). Crude mean level of T-cell p-p38 and their 95% confidence intervals (95%CI) are listed in the left part of the table, while adjusted coefficients (coef.), p-values (p) and 95% confidence intervals (95%CI) are listed in the right part of the table.

| | n (%) | Crude | | Adjusted | | |
|---|---|---|---|---|---|---|
| | | Mean | 95% CI | coef. | p | 95% CI |
| Gender | | | | | | |
| Female | 127 (42) | 755 | (714, 796) | ref | | |
| Male | 173 (58) | 784 | (752, 817) | -7 | 0.80 | (-47, 61) |
| Age (years) | | | | | | |
| [18, 39] | 112 (37) | 776 | (736, 816) | ref | | |
| [40, 49] | 79 (26) | 796 | (741, 851) | 17 | 0.60 | (-47, 81) |
| [50, 67] | 109 (37) | 751 | (710, 791) | -22 | 0.47 | (-81, 37) |
| Smoking status | | | | | | |
| Non-smokers | 207 (69) | 749 | (719, 779) | ref | | |
| Smokers | 93 (31) | 824 | (777, 871) | **73** | **0.01** | **(18, 129)** |
| Geographic[A] | | | | | | |
| Inhabitant >20km | 104 (35) | 779 | (741, 817) | ref | | |
| Inhabitant <6km | 104 (35) | 738 | (691, 786) | -28 | 0.37 | (-90, 34) |
| Employees | 92 (30) | 803 | (757, 849) | 15 | 0.66 | (-50, 79) |
| BMI | | | | | | |
| Low [<-, 25) | 106 (35) | 744 | (701, 789) | ref | | |
| Medium [25, 30) | 133 (45) | 780 | (742, 818) | 29 | 0.32 | (-29, 87) |
| High [30, ->] | 61 (20) | 803 | (749, 857) | 42 | 0.27 | (-33, 117) |
| hsCRP[B] | | | | | | |
| Low [0, 2] | 215 (72) | 757 | (728, 786) | ref | | |
| Medium [3, 5] | 52 (17) | 789 | (721, 856) | 29 | 0.41 | (-40, 98) |
| High [6-21] | 33 (11) | 843 | (763, 923) | 70 | 0.11 | (-15, 156) |

A) Described in Fig. 1a
B) 1 individual excluded due to missing CRP data.

**Table S4.** Effects on mean monocyte p-p38 from gender, age, smoking status, geographic group, body mass index (BMI) and tear film stability (NIBUT) (n=284). Crude mean level of monocyte p-p38 and their 95% confidence intervals (95%CI) are listed in the left part of the table, while adjusted coefficients (coef.), p-values (p) and 95% confidence intervals (95%CI) are listed in the right part of the table.

| | n | Crude MFI | | Adjusted | | |
|---|---|---|---|---|---|---|
| | | Mean | 95% CI | coef. | p | 95% CI |
| Gender | | | | | | |
| Female | 120 | 15,074 | (13,841, 16,307) | ref | | |
| Male | 164 | 15,262 | (14,251, 16,272) | -529 | 0.54 | (-2,224, 1,166) |
| Age (years) | | | | | | |
| [18, 39] | 102 | 15,952 | (14,661, 17,242) | ref | | |
| [40, 49] | 77 | 14,728 | (13,205, 16,251) | -1,146 | 0.26 | (-3,142, 850) |
| [50, 67] | 105 | 14,769 | (13,473, 16,064) | -988 | 0.29 | (-2,823, 847) |
| Smoking status | | | | | | |
| Non-smokers | 192 | 14,944 | (13,968, 15,920) | ref | | |
| Smokers | 92 | 15,680 | (14,390, 16,970) | 700 | 0.42 | (-988, 2,388) |
| Geographic[A] | | | | | | |
| Inhabitant >20km | 95 | 13,807 | (12,414, 15,200) | ref | | |
| Inhabitant <6km | 103 | 15,329 | (14,063, 16,596) | 1,555 | 0.11 | (-328, 3,438) |
| Employees | 86 | 16,526 | (15,146, 17,906) | **2,779** | **0.009** | **(712, 4,854)** |
| BMI | | | | | | |
| Low [<-, 25) | 100 | 14,397 | (13,078, 15,716) | ref | | |
| Medium [25, 30) | 128 | 15,290 | (14,191, 16,389) | 1,112 | 0.22 | (-670, 2,893) |
| High [30, ->] | 56 | 16,340 | (14,358, 18,323) | **2,295** | **0.04** | **(100, 4,490)** |
| NIBUT[B] | | | | | | |
| Continuous (7-60s) | | | | -8 | 0.73 | (-53, 38) |

A) Described in Fig. 1a
B) 17 individuals excluded due to missing NIBUT data.

**Table S5.** Effects on mean monocyte p-p38 from gender, age, smoking status, geographic group, body mass index (BMI) and airway obstruction (n=273). Airway obstruction was defined as a FEV1/FVC ratio below 0.7. Crude mean level of monocyte p-p38 and their 95% confidence intervals (95%CI) are listed in the left part of the table, while adjusted coefficients (coef.), p-values (p) and 95% confidence intervals (95%CI) are listed in the right part of the table.

| | n | Crude MFI | | Adjusted | | |
|---|---|---|---|---|---|---|
| | | Mean | 95% CI | coef. | p | 95% CI |
| Gender | | | | | | |
| Female | 112 | 15,222 | (13,933, 16,511) | ref | | |
| Male | 161 | 15,163 | (14,151, 16,176) | -676 | 0.44 | (-2,390, 1,039) |
| Age (years) | | | | | | |
| [18, 39] | 99 | 16,016 | (14,664, 17,368) | ref | | |
| [40, 49] | 72 | 14,793 | (13,237, 16,349) | -940 | 0.36 | (-2,975, 1,095) |
| [50, 67] | 102 | 14,662 | (13,380, 15,944) | -807 | 0.40 | (-2,709, 1,095) |
| Smoking status | | | | | | |
| Non-smokers | 187 | 14,896 | (13,918, 15,874) | ref | | |
| Smokers | 86 | 15,821 | (14,459, 17,184) | 1,020 | 0.26 | (-747, 2,787) |
| Geographic[A] | | | | | | |
| Inhabitant >20km | 96 | 13,545 | (12,182, 14,907) | ref | | |
| Inhabitant <6km | 90 | 15,733 | (14,372, 17,095) | **2,262** | **0.03** | **(318, 4,205)** |
| Employees | 87 | 16,436 | (15,067, 17,805) | **3,006** | **0.003** | **(1,004, 5,008)** |
| BMI | | | | | | |
| Low [<-, 25) | 94 | 14,403 | (13,014, 15,793) | ref | | |
| Medium [25, 30) | 121 | 15,252 | (14,137, 16,368) | 1,172 | 0.21 | (-652, 2,997) |
| High [30, ->] | 58 | 16,324 | (14,415, 18,232) | **2,251** | **0.045** | **(52, 4,450)** |
| Obstruction[B] | | | | | | |
| No | 239 | 15,157 | (14,306, 16,007) | ref | | |
| Yes | 34 | 15,403 | (13,114, 17,692) | -211 | 0.87 | (-2,718, 2,297) |

A) Described in Fig. 1a
B) 28 individuals excluded due to missing spirometry data

**Table S6.** Effects on mean monocyte p-p38 from gender, age, smoking status, geographic group, body mass index (BMI) and subjective stressful life events (IES-R) (n=301). Crude mean level of monocyte p-p38 and their 95% confidence intervals (95%CI) are listed in the left part of the table, while adjusted coefficients (coef.), p-values (p) and 95% confidence intervals (95%CI) are listed in the right part of the table.

| | n (%) | Crude MFI | | Adjusted | | |
|---|---|---|---|---|---|---|
| | | Mean | 95% CI | coef. | p | 95% CI |
| Gender | | | | | | |
| Female | 128 (43) | 15,297 | (14,093, 16,502) | ref | | |
| Male | 173 (57) | 15,207 | (14,241, 16,175) | -647 | 0.43 | (-2,270, 976) |
| Age (years) | | | | | | |
| [18, 39] | 112 (37) | 16,213 | (14,973, 17,453) | ref | | |
| [40, 49] | 79 (26) | 14,668 | (13,182, 16,156) | -1,475 | 0.13 | (-3,396, 444) |
| [50, 67] | 110 (37) | 14,676 | (13,436, 15,917) | -1,295 | 0.15 | (-3,065, 474) |
| Smoking status | | | | | | |
| Non-smokers | 208 (69) | 15,020 | (14,091, 15,948) | ref | | |
| Smokers | 93 (31) | 15,752 | (14,468, 17,036) | 666 | 0.43 | (-986, 2,318) |
| Geographic[A] | | | | | | |
| Inhabitant >20km | 104 (35) | 13,878 | (12,563, 15,192) | ref | | |
| Inhabitant <6km | 105 (35) | 15,505 | (14,239, 16,772) | 1,565 | 0.09 | (-263, 3,392) |
| Employees | 92 (30) | 16,497 | (15,183, 17,810) | **2,676** | **0.007** | **(736, 4,616)** |
| BMI | | | | | | |
| Low [<-, 25) | 106 (35) | 14,659 | (13,372, 15,945) | ref | | |
| Medium [25, 30) | 133 (44) | 15,261 | (14,194, 16,327) | 887 | 0.31 | (-842, 2,615) |
| High [30, ->] | 62 (21) | 16,218 | (14,356, 18,081) | 1,844 | 0.08 | (-250, 3,938) |
| Total IES-R | | | | | | |
| Low (< 24) | 290 (96) | 15,203 | (14,436, 15,969) | ref | | |
| High (> 23) | 11 (4) | 16,388 | (11,835, 20,941) | 768 | 0.71 | (-3,249, 4,785) |

A) Described in Fig. 1a